



# A Pilot Study Comparing Two Benthic Macroinvertebrate Collection Methods for Bioassessment of Wadeable Streams

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## ABSTRACT

This study compared the results of collecting and analyzing macroinvertebrate data using a composite versus a three single sample method. It was conducted as part of the Environmental Monitoring and Assessment Program (EMAP) Indicator Development Project of the U.S. Environmental Protection Agency (U.S. EPA). Data were collected from four sites on Mill Creek, which flows through the industrial heart of Cincinnati, Ohio, and one reference site on nearby Tanners Creek in southeastern Indiana. Samples were collected from nine transects and composited by habitat. Riffle/Run and Pool/Glide samples were composited separately and processed using U.S. EPA's EMAP macroinvertebrate sampling and laboratory protocols. A single sample also was collected at three randomly selected transects of the nine, but the three samples were processed in the laboratory as separate samples (not composited). Macroinvertebrate samples were analyzed using the Stream Benthos Integrity Index (SBII), consisting of 10 metrics. The data indicated that similar results were obtained using either sampling method. The SBII scores for the sites were ranked in the same order using either method, and these rankings were according to expectations based on chemical and physical conditions. It was concluded from the data that collecting and processing three random single samples from each stream reach gave similar SBII scores as the composite samples. The three single sample method also allows a measure of repeatability which gave more confidence in the results of the sampling data. The collection effort was 1/3 that required for the composite method and in this study the sorting and identification effort was also considerably reduced. It is recommended that additional studies be conducted on other gradient streams in other ecoregions to determine if the three sample method might reduce effort and expense in bioassessment studies elsewhere.

## INTRODUCTION

Macroinvertebrate sampling protocols have been designed for the purpose of evaluating the biological integrity of wadeable streams. These protocols were used in assessing the status of Appalachian streams, detecting stressors on community structure, and in assessing the relative severity of these stressors. This study was conducted as part of the Environmental Monitoring and Assessment Program (EMAP) Indicator Development Project of the U.S. Environmental Protection Agency (USEPA) to determine if a more efficient macroinvertebrate protocol could reduce effort and provide similar repeatable sampling data and reduce cost in processing samples. The sites selected for this comparison were in Mill Creek which flows through the industrial heart of Cincinnati, Ohio and Tanners Creek in southeastern Indiana.

## OBJECTIVES

To compare the results of collecting and analyzing the macroinvertebrate data using the composite versus the single sample collecting method.

## MATERIALS AND METHODS

Macroinvertebrate samples were collected in 1994 by kick net (Figure 1) from 5 sites (MC-001 Hamilton/Mason Road Site, MC-002 Windisch Road Site, MC-003 Pristine Landfill Site, MC-005 Ridgewood Arsenal Site on Mill Creek) and (TC-001) on nearby Tanners Creek (Figure 2). At each site eleven transects were systematically marked off equal distances apart in proportion to stream size. The composite macroinvertebrate samples were collected from 9 inner transects as follows: At the first transect (most downstream transect) a sample was collected randomly for either the left, center, or right points of the stream. The additional eight transects were then sampled left, center, or right systematically. All the riffle and pool samples were composited separately so that two composite samples were collected from each stream reach. The samples were collected from riffles/runs by holding the kick net securely while kicking the substrate within a 0.5 m<sup>2</sup> area, vigorously for 20 seconds or in pools/glides by kicking the substrate vigorously and dragging the net repeatedly through the disturbed area just above the bottom while continuing to kick for 20 seconds. The composite samples were collected and analyzed using the U.S. Environmental Protection Agency, Environmental Monitoring and Assessment Project protocols (Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams, EPA/620/R-94/004F, 1998) for 300 organism counts. The single samples were collected and analyzed in the same way except the three samples were collected at each of three randomly selected transects from which the composite samples were collected. These single samples were processed separately (not composited).

The macroinvertebrate data analyses used ten metrics including taxa richness, composition, and pollution tolerance measures to generate a Stream Benthos Integrity Index (SBII) score to determine the biological condition of the sampling stations (Tables 1 and 2). The ten metrics used were: Total Taxa, HBI, Number Individuals per Taxon, % Intolerant Taxa, % Non-Insects, % Chironomidae, % Individuals in Dominant Taxon, % EPT Taxa, EPT Index, and % Oligochaetes and Leeches. Data were used to generate species lists, taxa composition, and diversity metrics. Stream Benthos Integrity Index scores and biological conditions (Table 3) were used to evaluate the performance of the sites in Mill Creek and Tanners Creek (reference site).

Water and sediment temperatures and water chemistries (pH, alkalinity, hardness, conductivity, and D.O.) were taken in June, 1994 at each site (Table 4). Sediment samples from each site were analyzed for metals (Table 5), using standard chemical methods and QC elements.

All the sorted organisms were identified to the lowest taxon possible and enumerated. Taxonomic quality assurance and quality control were maintained by using a variety of literature and taxonomic guides for identification and other standard QC procedures.

Differences between sample methods, single sample versus composite, were examined by two way ANOVA with sample method and site as factors. ANOVAs were run separately for Number of Taxa (Table 6) and SBII scores (Table 7).

FIGURE 1. MODIFIED KICK NET (595/600 µm MESH)

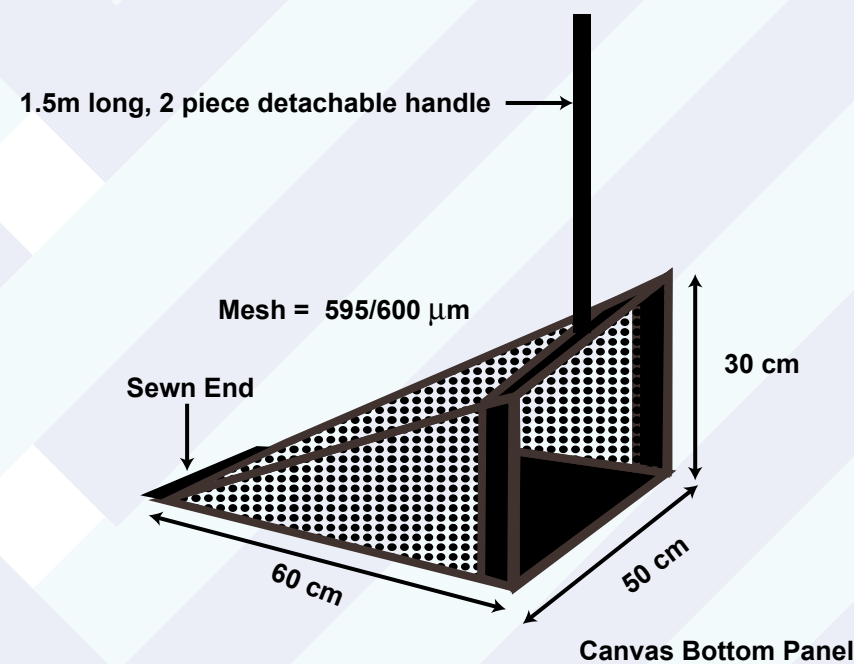


FIGURE 2. LOCATIONS OF SITES STUDIED.

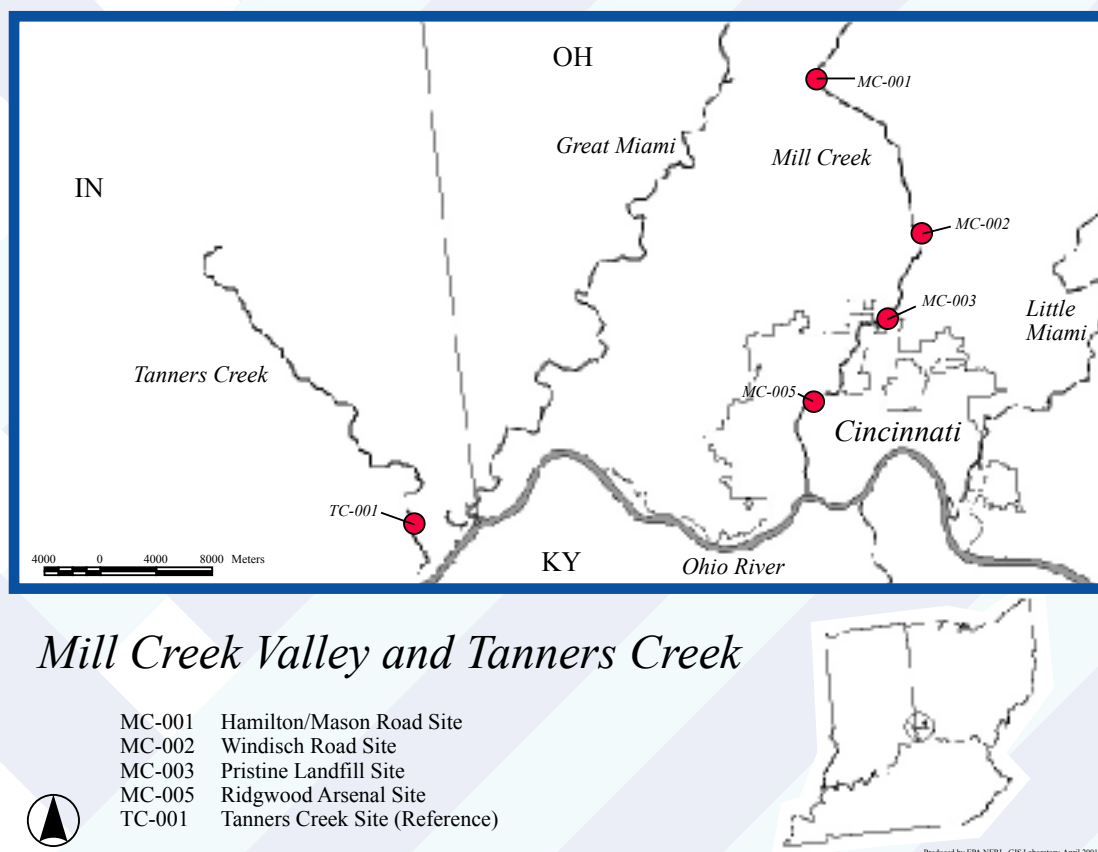


TABLE 1. PROPOSED 10 METRICS FOR COMPOSITE RIFFLE/RUN SAMPLES.

Metric	5	4	Value 3	2	1
1. Number of Taxa	> 30	20 - 30	10 - 19	5 - 9	< 5
2. HBI	< 3.0	3.1 - 4.5	4.6 - 6.0	6.1 - 8.0	> 8.0
3. No. Individ./Taxon	< 4.0	4.0 - 6.9	7.0 - 10.0	10.1 - 20.0	> 20.0
4. % Intolerant Taxa	> 40	26 - 40	10 - 25	1 - 10	0
5. % Non-insects	< 20	20 - 39	40 - 69	70 - 90	> 90
6. % Chironomids	< 20	20 - 25	26 - 35	36 - 50	> 50
7. % Ind. Dom. Taxon	< 20	20 - 35	36 - 50	51 - 80	> 80
8. % EPT Taxa	> 50	26 - 50	11 - 25	1 - 10	0
9. EPT Index	> 25	11 - 25	5 - 10	1 - 4	0
10. % Oligo. & Leeches	0	1 - 4	5 - 7	8 - 10	> 10

The total SBII score is used as follows to determine biological condition:

Very Poor	Poor	Fair	Good	Excellent
0 - 16	17 - 25	26 - 32	33 - 41	42 - 50

Nominal (33 - 50); Marginal (26 - 32); Subnominal (0 - 25)

Note: If there are fewer than 5 individuals in the sample, the site has a SBII score of 0.

TABLE 2. PROPOSED 10 METRICS FOR COMPOSITE POOL/GLIDE SAMPLES.

Metric	5	4	Value 3	2	1
1. Number of Taxa	> 30	20 - 30	10 - 19	5 - 9	< 5
2. HBI	< 3.0	3.1 - 4.5	4.6 - 6.0	6.1 - 8.0	> 8.0
3. No. Individ./Taxon	< 4.0	4.0 - 6.9	7.0 - 10.0	10.1 - 20.0	> 20.0
4. % Intolerant Taxa	> 40	26 - 40	10 - 25	1 - 10	0
5. % Non-insects	< 20	20 - 39	40 - 69	70 - 90	> 90
6. % Chironomids	> 50	36 - 50	26 - 35	20 - 25	< 20
7. % Ind. Dom. Taxon	< 20	20 - 35	36 - 50	51 - 80	> 80
8. % EPT Taxa	> 50	26 - 50	11 - 25	1 - 10	0
9. EPT Index	> 25	11 - 25	5 - 10	1 - 4	0
10. % Oligo. & Leeches	0	1 - 4	5 - 7	8 - 10	> 10

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Nominal (33 - 50); Marginal (26 - 32); Subnominal (0 - 25)

Note: If there are fewer than 5 individuals in the sample, the site has a SBII score of 0.

BORGMAN'S WORLD



TABLE 3. MILL CREEK AND TANNERS CREEK SITE DATA

Site TC001	Single Pool	Single Pool	Single Pool	Composite Riffle (1)	Composite Pool(8)
Individuals	88	300	318	209	323
Taxa	13	27	32 (**52)	25	34 (**47)
SBII Score	34(good)	38(good)	35(good)	43(excellent)	36(good)
Site MC001	Single Riffle	Single Pool	Single Pool	Composite Riffle (3)	Composite Pool (6)
Individuals	55	165	131	299	288
Taxa	18	25	19 (*34)	44	36 (**57)
SBII Score	31(Fair)	32(fair)	32(fair)	35(good)	32(fair)
Site MC002	Single Pool	Single Pool	Single Pool	Composite Riffle (1)	Composite Pool (8)
Individuals	41	267	246	302	284
Taxa	17	28	8 (*32)	25	24 (**35)
SBII Score	34(good)	29(fair)	18(poor)	29(fair)	27 (fair)
Site MC003	Single Riffle	Single Riffle	Single Pool	Composite Riffle (5)	Composite Pool (4)
Individuals	329	66	84	302	242
Taxa	32	20	16 (*41)	28	46 (**52)
SBII Score	25(poor)	29(fair)	22(poor)	26(fair)	30(fair)
Site MC005	Single Riffle	Single Riffle	Single Pool	Composite Riffle (5)	Composite Pool (4)
Individuals	114	105	74	339	86
Taxa	23	20	20 (*38)	36	20 (**41)
SBII score	24(poor)	25(poor)	29(fair)	31(fair)	26(fair)

\*Total Number of Taxa in All Single Samples

\*\* Total Number of Taxa in Composite Samples

TABLE 4. WATER CHEMISTRIES; WATER AND SEDIMENT TEMPERATURES FROM MILL CREEK & TANNERS CREEK

Sample	Temp. (°C) Sed.	Temp. (°C) Water	pH (S.U.) Water	Alkal. (ppm) Water	Hard. (ppm) Water	Cond. (µS/cm) Water	D.O. (ppm) Water
MC001	20.0	22.0	8.23	256	388	678	9.0
MC002	20.1	23.1	8.01	230	300	556	8.8
MC003	20.1	22.1	8.13	176	246	668	9.1
MC004	20.1	22.1	8.13	176	246	668	9.1
MC005	20.1	21.1	8.15	164	222	535	9.1
TC001	20.1	22.1	8.10	120	166	320	9.1

TABLE 5. SEDIMENT CHEMISTRY - (METALS) & TOTAL ORGANIC CARBON (TOC)

ANALYTE	MC001	MC002	MC003	MC004	MC005	TC001
TOC (g/kg)	20.50	13.20	8.07	11.90	5.98	4.43
Mercury (mg/kg)	0.088	0.005	0.017	0.001	0.026	0.0001
Aluminum (mg/kg)	17.3	22.1	22.8	21.2	25.0	13.9
Chromium (mg/kg)	16.8	14.7	31.1	24.0	28.7	19.0
Copper (mg/kg)	16.5	7.77	14.3	25.3	22.0	15.7
Iron (g/kg)	24.6	14.7	16.6	22.4	14.9	27.7
Lead (mg/kg)	14.9	16.5	24.9	32.0	45.5	22.5
Manganese (g/kg)	1.03	0.583	0.448	0.614	0.455	0.981
Nickel (mg/kg)	12.2	9.6	18.0	13.4	23.5	11.1
Zinc (mg/kg)	21.2	28.6	74.4	46.5	63.4	25.0
Relative Conc. **	124.6	114.6	382.3	185.4	223.5	135.9
Zn+Pb+Cu+Cr+Hg (mg/kg)	69.5	67.7	144.7	127.8	159.6	82.2

\*\* relative concentration. Concentrations were not all converted to the same units (raw numbers in the above rows were added) because the object is to assess relative contamination of sites, and certain less toxic metals (such as Fe, Mn) would dominate the total if all concentrations were converted to the same units. Total is for elements only (TOC not included).

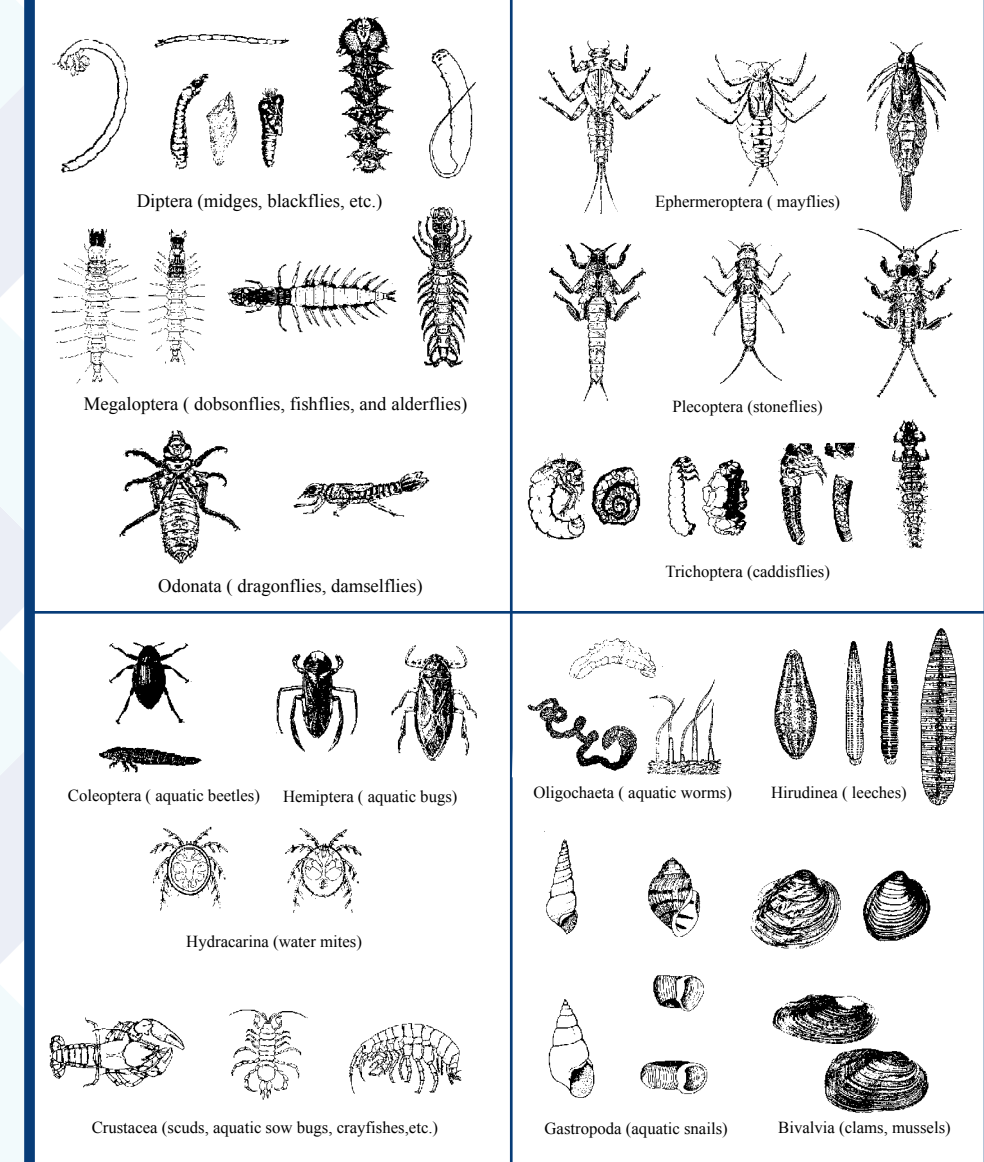
TABLE 6. NUMBER OF TAXA (MEAN ± SD)

Site	Composite	Single
MC001	40 ( 5.7)	21 ( 3.8)
MC002	25	18 ( 10 )
MC003	37 ( 13 )	23 ( 8.3)
MC005	28 ( 11 )	21 ( 1.7)
TC001	30 ( 6.4)	24 ( 9.8)

TABLE 7. SBII SCORES (MEAN ± SD)

Site	Composite	Single
MC001	33.5 ( 2.1)	31.7 ( 0.6)
MC002	29	27.0 ( 8.2)
MC003	28.0 ( 2.8)	25.3 ( 3.5)
MC005	28.5 ( 3.5)	26.0 ( 2.6)
TC001	39.5 ( 4.9)	35.7 ( 2.1)

## BENTHIC MACROINVERTEBRATES



## RESULTS AND DISCUSSION

Selected chemical water quality characters of the Mill Creek sites and Tanners Creek reference site are shown in Table 4. These data indicated little difference between the impacted Mill Creek sites and the Tanners Creek site except varying differences in water hardness and conductivity.

Sediment metal chemistry results (Table 5) indicated several sites containing elevated concentrations of a variety of metals. The samples from sites MC003 and MC005 exhibited the most numerous elevated concentrations of metals, including zinc, copper, cadmium, chromium, lead.

The 10 metrics and values for the 10 metrics that were used in determining the Stream Benthos Integrity Index (SBII) are shown in Table 1 and 2. Table 3 shows the total individuals, total taxa, SBII scores and biological conditions for the three single and the two composited samples collected at each site.

Although six of the 15 single samples contained less than 100 organisms, the SBII scores were similar for both the single and composite samples containing more than 100 individuals. As would be expected, most of the single samples contained fewer taxa (Table 6) than the composited samples and the differences were significant (p < 0.01). However, this did not result in significantly different SBII scores (Table 7), using either sampling method (p = 0.1052). The mean SBII scores for the sites were ranked in the same order using either method, and these rankings were as expected based on chemical and physical conditions (Tables 4 and 5). The differences were not significant perhaps due to the wide range of variability and the small sample size.

We concluded that collecting and processing three random single samples from each stream reach gave the same SBII scores as collecting nine samples using the composite method. The three sample methods also allows a measure of repeatability which gave more confidence in the results of the sampling data.

The collection effort for the single sample method was 1/3 that required for the composite method and in this study the sorting and identification effort was considerably reduced due to much less debris and often fewer organisms to identify.

## FUTURE WORK

While the single sampling collecting method and the composite sampling method appeared to be equally reliable in assessing stream biological conditions, additional studies need to be conducted to determine how widely these conclusions can be applied. Conclusions reached in this study appear encouraging as a means of reducing the cost of sampling and analysis procedures.